## Phylogeny

ULK3 is a member of the Unc-51-like kinase (ULK) family within the serine/threonine protein kinase group of the human kinome (Preuss et al., 2020). Phylogenetic analyses cluster ULK3 together with the STK36/Fused sub-family, sharing ~38 % catalytic-domain identity with human STK36 and ~37 % with Drosophila fused kinase (Maloverjan et al., 2010a). Within the ULK family, ULK3 forms a branch distinct from ULK1/2/4, linking it to Hedgehog rather than canonical autophagy signalling (Karmacharya & Jung, 2023).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (Maloverjan et al., 2010a).

## Cofactor Requirements

Catalytic activity requires divalent cations; Mg²⁺ or Mn²⁺ support activity in vitro (Kasak et al., 2018).

## Substrate Specificity

ULK3 phosphorylates GLI transcription factors, showing highest activity toward GLI2 and lower activity toward GLI1 and GLI3 (Maloverjan et al., 2010a). On GLI1, phosphorylation is confined to residues 1–426 (N-terminus) and the C-terminal region, with the central segment (426–754) unmodified (Maloverjan et al., 2010a). No global consensus phosphorylation motif has been reported.

## Structure

The 472-residue protein contains an N-terminal kinase domain (residues 14–270), a microtubule-interacting and trafficking (MIT) domain (279–353) and a C-terminal regulatory tail (354–472) (Maloverjan et al., 2010a). Canonical VAIK (Lys44), HRD and DFG motifs constitute the catalytic core; a second essential lysine (Lys139) is also required—mutation K139R abrogates activity (Kasak et al., 2018; Maloverjan et al., 2010a). Homology modelling indicates at least six surface-exposed autophosphorylated serines near the active site (Kasak et al., 2018). No crystal or cryo-EM structure is currently available; structural insights rely on homology models (Kasak et al., 2018).

## Regulation

• Autophosphorylation occurs at Ser22, Ser55 (kinase domain) and Ser300, Ser350, Ser384, Ser464 (C-terminal tail) (Kasak et al., 2018; Maloverjan et al., 2010b).  
• Ser22/Ser55 autophosphorylation is dispensable for catalysis, whereas phosphorylation by an unidentified kinase at Ser176 and Ser467/468 abolishes activity (Kasak et al., 2018).  
• In the absence of Sonic Hedgehog (SHH), Suppressor of Fused (SUFU) binds the kinase domain and blocks both auto- and substrate phosphorylation; SHH stimulation dissociates the SUFU–ULK3 complex, permitting activation and GLI2 phosphorylation (Maloverjan et al., 2010b).  
• SUFU-bound ULK3 also recruits PKA, GSK3β and CK1 to promote GLI2 repressor processing in a kinase-independent scaffolding role (Maloverjan et al., 2010b).  
• The small molecule SU6668 binds the ATP pocket and inhibits ULK3 in a mixed-type manner, increasing Km and decreasing Vmax for ATP (Kasak et al., 2018).

## Function

Highest ULK3 mRNA levels are detected in fetal brain and in adult hippocampus, cerebellum, olfactory bulb and optic nerve (Maloverjan et al., 2010a). Active ULK3 enhances GLI1/GLI2 transcriptional activity and promotes nuclear localisation of GLI1, positively regulating Hedgehog signalling (Maloverjan et al., 2010a). Partial knock-down paradoxically augments SHH-induced GLI1, indicating dual positive/negative pathway modulation (Maloverjan et al., 2010b). ULK3 is up-regulated in cancer-associated fibroblasts under metabolic stress, where it is essential for autophagy-driven pro-tumorigenic conversion (Karmacharya & Jung, 2023). Verified interactors include SUFU (kinase domain) and GLI1-3 (kinase and MIT regions) (Maloverjan et al., 2010a; Maloverjan et al., 2010b).

## Inhibitors

SU6668 photo-crosslinks to Ile43 in the ATP pocket and inhibits ULK3 activity; a photoreactive analogue (SUX) covalently binds the same site. SU6668 suppresses SHH-induced GLI expression in cells (Kasak et al., 2018; Montagnani & Stecca, 2019).

## Other Comments

Elevated ULK3 activity in cancer-associated fibroblasts highlights the kinase as a potential therapeutic target; no recurrent disease-linked mutations have been reported (Karmacharya & Jung, 2023; Maloverjan et al., 2010a).

## 9. References

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